# ADJUSTMENT OF REACTION OF CULTURE MEDIUMS

ERIC A. FENNEL AND MAUD B. FISHER

*Army Medical School, Washington, D. C.*

For the titration of culture mediums, the use of phenolphthalein as an indicator, determining the total acidity, is rapidly becoming obsolete. This does not apply, without qualification, to the mediums for the growth of B. typhosus, B. paratyphosus, staphylococcus and such other organisms that have a wide range of selectivity in cultural requirements; it does, however, apply to the pneumococcus, streptococcus, B. influenzae, meningococcus and the more strictly parasitic organisms that have a comparatively narrow range of selectivity in cultural requirements.

These facts were forcibly brought to our attention when it developed on us to produce large quantities of pneumococci in a serum-free broth. It has been the custom to grow pneumococci in infusion broth that has been titrated to + 0.2 (0.2% acid) to phenolphthalein, the titration being done at the boiling point, and a very faint shade of pink being considered the neutrality point. It is very difficult for even the experienced worker to determine accurately this neutrality point, and in a highly colored broth, the difficulty is even greater.

Hiss and Zinsser say that "the most favorable reaction of media for the cultivation of this organism (pneumococcus) is neutrality, or moderate alkalinity (two-tenths to eight-tenths per cent. alkalinity to phenolphthalein). Slight acidity, however, if not exceeding eight-tenths per cent., does not materially hamper development."

Beef infusion broths were therefore made accordingly, but while some batches of broth gave good results, others partially or wholly failed to grow the pneumococcus. Batches of greater alkalinity were tried—these gave very poor results; batches of greater acidity were tried, and while the results were better, great irregularity of growth was encountered.

The method of titrating the broth by the hydrogen-ion concentration method was then tried, and found to be wholly practical and simple. Once the proper degree of alkalinity, measured in terms of hydrogen-ion concentration (which is more simply expressed by the

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symbol $P_H$) was determined, profuse and consistent growth of the pneumococcus was secured. When this optimum reaction for the pneumococcus had been found to be $P_H$ 7.8 to 8.0, this broth was found to be + 3.0 to + 3.5 (3-3.5% acid) to phenolphthalein, a total acidity far beyond anything previously suggested for the growth of pneumococcus. Our experience with the meningococcus was similar to our experience with the pneumococcus. A glucose, beef infusion agar, titrated to neutrality with phenolphthalein, as well as slight degrees to either side of the neutrality point, failed to give us luxuriant growth with any degree of consistency. It was only when such an agar had been set in reaction by the $P_H$ method that profuse growth could be obtained with absolute regularity.

Much has been written of hydrogen-ion concentration during the past few years.[1] This has been, in the main, couched in highly technical terms of chemistry and mathematics. Shorn of this impressive verbiage, this system of titration resolves itself into a simple, highly practical and accurate method. It is true that a complete understanding of the underlying principles demands close study and application; it is further true that if one were interested in all the reactions of substance, ranging from a normal HCl on one side to ($P_H$ 0.0) to a normal NoOH ($P_H$ 14.0) on the other, it would necessitate the possession of a large number of solutions or reagents. It is still further true that the bacteriologist is interested only in the neutrality point and in those reactions lying just to either side. Bearing in mind that a $P_H$ of 7 is absolute neutrality, and that 7.3-7.5 is the approximate reaction of normal blood, it is seen that a range of reactions from 6.6-8.4 is all that is necessary, for practical purposes, in bacteriology.

To set the reaction of a broth is a simple matter. A measured 10 c c of the broth are taken in a test tube that has been rinsed with a portion of this broth. One-half c c of a 0.02% solution of phenolsulphonephthalein—frequently referred to as phenol red—is added, and from a burette a measured quantity of N/20 NaOH is added until the color matches the standard chosen. Simple calculation then determines the amount of N/1 to be added to the entire quantity of broth to bring it to the reaction of the chosen standard. This standard—say 7.8—consists of a solution of $KH_2PO_4$ and NaOH in distilled water with phenol red. This water is double distilled and the combination of pure salts is very accurately weighed out. It is the preparation of these standards (a graded solution of these salts with color indicator for each $p_H$ desired, about 8-10 being necessary for bacteriologic work) that has heretofore presented the greatest difficulty.

Under ideal conditions these standard solutions would be made up daily, with specially distilled water, with salts of greatest purity, very accurately weighed out, and the complete salt solutions, or standard, tested for $P_H$ accuracy by an electrometric method.

If a chemist is available, it is possible to have him prepare the 10 different salt solutions, ranging from 6.8-8.4, each in a quantity of 200 c c, and these

[1] Tizard: Brit. Assn. Report, 1911, p. 268; Washburn: Principles of Physical Chemistry, p. 333; Clark and Lubs: Bacteriol., 1917, 2, p. 1. Sörensen: Biochem. Zeitschr. 1909, 21, p. 131. Findlay: Practical Physical Chemistry, p. 187 et seq.

solutions, with proper care, could be used over an extended period of time. The weights and measures for these standards are appended.

To make color standards of these solutions it is necessary only to measure 10 c c of each solution into a chemically clean test tube, add 0.5 c c of the 0.02% solution of phenol red, and cork the tube. The material under titration is brought to the same color shade as the color standard that represents the desired $P_H$.

The fact that commercial laboratories[2] are now preparing, at a reasonable price, these standard color solutions, sealed in ampules, and sufficient phenol-sulphonephthalein for extended use, makes the entire process so simple, and so much more accurate than the old-fashioned phenolphthalein titration, that there is no longer an excuse for any laboratory, no matter how small or isolated, to neglect this method.

The ampuled standards present a graded series of shades of yellow, pink and red; deep red appears on the alkaline or 8.4 side, and pale yellow to faint pink on the acid or 6.6 side. These standards may be used with safety for about 6 months, during which time, when not in use, they are to be kept in the dark at an even temperature.

Such a series of ampules, along with a combined rack and comparator are prepared at the Army Medical School and issued for use in the service.

When a medium of a given $P_H$ is desired, the standard color ampule of the desired $P_H$ is selected and set aside. Into a tube of the same diameter a measured 10 c c of the ampule, rinsed out with the medium to be titrated, are placed and to this are added 0.5 c c of the solution of phenol red. From a burette a solution of NaOH, known to be 1/20 strength of a concentrated solution, is added until the color deepens and finally matches the standard selected.

In order that the native color of the medium under titration be discounted, a simple comparator is used. It is merely a block of wood with 4 holes to receive 4 test tubes, and with 2 horizontal holes, each piercing 2 of those that receive the tubes. A dipping in black paint improves it. In the 2 front holes are set the tubes of the medium to be titrated. To the tube on the left, which contains 10 c c of this medium are added 0.5 c c of the phenol red solution and the contents mixed by a swirling motion. Behind this tube on the left is set a tube of distilled water; behind the tube on the right is set the standard to which it is desired to adjust the broth. In this fashion, a direct comparison may be made by glancing through the horizontal holes, the eye seeing on each side the same total contents; i. e., distilled water, color indicator, medium and glass. Dilute NaOH is now added from a burette, to the front tube on the left, until the colors match. Simple calculation determines the amount of concentrated NaOH, 20 times the strength of the dilute, necessary to bring the bulk of the medium to the proper adjustment. After the addition of this concentrated NaOH, it is best, after thoroughly stirring, to test a sample of the adjusted medium, by placing 10 c c in a tube, adding 0.5 c c of the indicator, phenol red, and placing in the comparator. Any deviation may be corrected by the addition of more NaOH or HCl, until the color matches absolutely.

It is convenient to use, for the stock 0.02% solution of phenol red, a bottle fitted with a perforated stopper, through which has been thrust a 1 c c pipet. This pipet is to be covered with a bit of tinfoil when not in use.

Daylight, direct or indirect, is to be used for comparisons; if artificial light is necessary, a nitrogen bulb and ground-glass screen should be used.

[2] Hynson, Westcott & Dunning, Baltimore, Md.. supply such standards and indicators.

When one has become accustomed to working with these color standards one can almost memorize the shades of color representing certain $P_H$'s much in use. If one should be wholly without standards, and have only a 0.02% solution of phenolsulphonephthalein (renal test ampules, containing 6 mg. are usually available), recourse may be had to a very valuable method brought out by Barnett and Chapmann.[3] A tube, containing 10 c c of a very weak acid (about N/40 HCl) and another containing 10 c c of a very weak alkali (about N/20 NaOH) have added to each 0.25 c c of the indicator solution, phenol red. The acid tube becomes quite yellow, the alkaline tube becomes quite red. Looking through both of these tubes simultaneously, a shade of pink representing a $P_H$ of 7.8 or 7.9 is seen.

Barnett and Chapmann have carried their idea further and have devised a method whereby it is possible to obtain approximate accuracy with the other degrees of hydrogen-ion concentration within the phenol red range.

Six tubes each containing 10 c c of the weak acid have added to them, in order, the following quantities of the phenol red solution: 0.45, 0.4, 0.35, 0.30, 0.25, 0.20 c c; six tubes of the weak alkali have added to them, in order, the complementary quantities, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 c c. Looking through the first pair of tubes (not mixing them) they state that the $p_H$ represented is 6.9 while the last pair of tubes represents 8.1. The entire range is 6.9, 7.2, 7.5, 7.7, 7.9, 8.1. Kligler[4] has determined the result to be 7.1, 7.3, 7.5, 7.7, 7.8, 7.9. It has been our experience that such combinations yield the following figures: 7.0, 7.3, 7.5, 7.7, 7.8, 8.0. The slight disparity in the results shows that while slight variations do occur and make the method not absolutely accurate, the error is so slight as to be of little importance in the adjustment of culture mediums.

The use of 2 tubes to make a color standard, would necessitate the use of a comparator, to receive 3 tubes instead of 2, a matter simple to rectify.

Liquid mediums, such as broth, may be titrated either hot or cold; cold is to be preferred, for it more closely simulates the conditions under which the bacteria will be grown.

Agar mediums are best adjusted in the broth stage, and the agar added as the last step. Agar is, for all practical purposes, neutral in reaction, and does not change the $p_H$ of the broth. If this should not be convenient, the agar may be melted and titrated hot. In comparing the colors with agar it is best to wait until the agar has set before concluding that the colors are identical. It is a simple matter to send this tubed specimen, with indicator in it, along with the batch of mediums, through the sterilization process to check the reaction after sterilization.

Relatively large quantities of blood serum, or whole blood, added to an alkaline or acid medium, tend to approximate the final $P_H$ to 7.3 or 7.5. Besides this, because of the balanced basic and acid salts contained in blood serum, such an addition of serum tends to hold stable the hydrogen-ion concentration of a medium, during bacterial growth. These salts are usually referred to as "buffer salts." The addition of blood serum may be imitated by the addition of about 0.5% $K_2HPO_4$ to a medium, thereby increasing its "buffer salt" content. An appreciable amount of acid must then be produced by the growing organisms before the medium becomes more acid. Over and above the advantage of the nutrient properties of the serum, this accounts in part for some of the evident superiority of blood mediums.

[3] Jour. Am. Med. Assn., 1918. 70. p. 1062.

[4] Jour. Bacteriol., 1919, 4, p. 35

The usual methods of sterilizing medium — the Arnold and the autoclave — serve to change the $P_H$ of mediums but slightly. We have found simple nutrient (extract) agar to change but 0.2 to 0.4 of a $P_H$. With the infusion mediums, in which there is a higher proportion of the so-called buffer salts—the natural salts of the meat—the change is usually about 0.2, though very frequently there is no change at all.

There is no constant interrelationship between $p_H$ readings and phenolphthalein titrations, except in a case where a definite relationship has been established, in a medium of absolutely standard construction, using distilled water, the same batch of meat extract and peptone. Any change in technic changes the relationship between the two systems of titration; as a matter of fact, the $p_H$ is so satisfactory and practical, that this relationship is only of academic interest.

It now develops that most of the organisms of interest to the bacteriologist have well defined limits of $p_H$ in which they will grow, as well as an optimum. This subject has received increasing attention during the past year, and it will not be long before the optimum and limits of all the important organisms will be well defined in terms of $p_H$.

*B. typhosus and B. paratyphosus.*—These have wide ranges of $p_H$ in which they will grow, and therefore an ill defined optimum. These organisms will grow on an agar as alkaline as $P_H$ 9.6, and on agar of an acidity of $P_H$ 4 (it would be better to say *in* agar, for at $P_H$ 4 agar will not solidify). The optimum ranges from 6.2-7.2.

Making such simple nutrient agar with Armour's extract and peptone, the final reaction, without adjustment, is usually $P_H$ 6.3. This is really ideal for the growth of B. typhosus. Such a 3% agar, at $P_H$ of 6.3 offers an excellent surface for inoculation; this same agar, at a $P_H$ of 4 will not solidify. At 4 it is fluid, at 4.2 it is thick and slushy, at 4.4 it is very soft, and much water of synerisis collects, 4.6, 4.8 and 5 present agars of soft consistency and crumbling surface. From 5.2 on, the agar becomes "workable." As one goes from 6.3 toward 4 the agar becomes softer and finally useless; as one goes from 6.3 toward 9.6 the agar becomes more and more cloudy, from precipitated phosphates, until at about 8 it becomes very unsatisfactory. Therefore a plain nutrient agar, or trypsin agar, of from 6.2-7-2 offers the best reaction for this medium measured by both the characteristics of the medium and its nutrient qualities.

What has been said for B. typhosus holds good for B. paratyphosus A and B, except that these organisms thrive better in the more alkaline reactions than does the typhoid bacillus. B. paratyphosus B, in addition, thrives much better in very acid mediums (4) than do the other two.

It might be of interest to note that the faculties of B. paratyphosus A and B, for fermenting minimal quantities of sugar, with gas production, seem also to have $P_H$ limits (for B. paratyphosus A from 5.4-7.8, and for B. paratyphosus B from 4.8-8.4), with the optimum, for these two organisms, with regard to gas formation, fairly well defined. For B. paratyphosus A this optimum is from 6.3-7.4; for B. paratyphosus B this optimum is from 5.2-7.6.

It is well to bear these facts in mind for the construction of Russell's double sugar. With this medium, on the lower side one is limited by a soft agar and a red medium, on the upper side one is limited by a too blue

medium. Kligler[5] has recommended $P_H$ 7.4 for Russell's double sugar medium. We have found either 7.4 or 7.6 to be very satisfactory, since this point gives optimum growth for typhoid, B. paratyphosus A and B. paratyphosus B, colon and dysentery and is well within the optimum gas range of the two latter organisms. At this $P_H$, also, litmus has the delicate lavender tint necessary for this medium. Kligler recommends $P_H$ 7.8-8.0 for the construction of Endo mediums.

Norton[6] has called attention to this subject of hydrogen-ion concentration adjustment estimation of culture mediums, and has collected some of the data that have appeared during the past year. Kligler recommends a $p_H$ of 7.0-7.2 for brilliant green agar. Meyer and Stickel[7] recommend a slightly more acid reaction for brilliant green mediums, 6.4-7, and for their peptic digest mediums, for typhoid bacillus, 7.0-7.2. The latter is within the range of phenol red, i. e., 6.6-8.4.

The members of the committee of the American Public Health Association[8] in their standard method of examining disinfectants, recommend $P_H$ 6.0-7.0, optimum at $p_H$ 6.5.

*The Pneumococcus.*—At the time we found it necessary to determine the optimum $P_H$ for the pneumococcus, the figures of Dernby and Avery[9] were not available. We found that the following broth gave excellent and consistent growth. One pound of ground beef in 1 liter of water is heated to 55 degrees for one hour, after which it is filtered through cotton. Armour's peptone, 1%, and NaCl, 0.5%, are then added, and the broth is brought to boiling to dissolve the peptone. The broth is then set to $P_H$ 7.8-8.0, after which it is filtered through paper. In liter quantities it is autoclaved 1 hour, and the reaction changes hardly at all. Records were kept on several hundred batches of broth, made up in this standard fashion. While the figures fluctuated slightly, the average run of broth presents the following data:

Native phenolphthalein reaction of broth = + 4.3%; native $p_H$ = 6.4.
Corresponding total acidity = + 2.4%; set to = 7.8
Autoclaved, 1½ liter lots, 15 lbs., 60 minutes
Total acidity immediately after autoclaving = + 2.7%; $p_H$ = 7.5.
Total acidity after 24 hours' incubation at 37 C. = 3.5%; $p_H$ = 7.8.

This broth gives rich and consistent growth of the pneumococcus and the addition of 0.5% glucose makes 5-7 billion per c c possible. We found that 8.2 was the alkaline limit, while 7.2 was about the acid limit. These facts are closely in accord with Dernby and Avery. While it is difficult to plant a glucose broth of $P_H$ 7.2 and consistently get growth, pneumococci, planted in a $P_H$ 7.8 glucose broth will continue to grow until they have produced a $P_H$ of about 5.2. This applies not only to types I, II and III, but also to type IV. There is reason to believe that this end point of acid production may be a differentiating factor in the streptococci; there is reason to hope that it may assist in differentiating pneumococci from streptococci.

*Streptococci.*—All the strains of Streptococcus viridans with which we have had to deal, have grown well in a $P_H$ 7.8-7.6 infusion broth, though some strains remain suspended in an even emulsion, others sediment out, in growth.

[5] Jour Exper. Med., 1918, 28, p. 319.
[6] Amer. Jour. Pub. Health, 1919, 9, p. 3.
[7] Jour. Infect. Dis., 1918, 23, p. 53.
[8] Amer. Jour. Pub. Health, 1918, 8, p. 506
[9] Jour. Exper. Med., 1918, 28. p. 345.

Blood agar, for pneumo- and streptococcus, may be made by adding agar to the preceding broth, and, after sterilization, the requisite amount of corpuscles, and consistent growth may be expected.

*Influenza Bacillus.*—The cultivation of the B. influenzae seemed to have offered considerable difficulty, early in the epidemic, and may be accountable for some of the widely varying reports. The development of the so-called "chocolate agar," an infusion agar, to which laked blood is added while the agar is 90 C., removed many of the difficulties. If this infusion agar be set to $p_H$ 7.8 or 8.0[10] rather than the untrustworthy 0.2% acid to phenolphthalein, even more regularity in results may be expected.

*B. Cholerae.*—This organism grows well on the ordinary extract agar, or broth, within a range from 5.6-9.6 +. Its optimum is about from 6.2-8.0.

*B. Dysenteriae.*—This organism grows well on plain agar, within a range from 5.4-9.6 +. Its optimum is about from 6.3-7.8. There seems to be no different selectivity on the part of the Shiga, Flexner or Y strains, except that Shiga does not grow quite so well in the more acid mediums.

*Meningococcus.*—Great difficulty was found in growing the meningococcus on a serum-free infusion glucose agar, titrated by phenolphthalein, when this organism was desired in large quantities for vaccine production. When the $P_H$ system of titration was substituted, the problem became comparatively simple. The meningococcus, on such a medium, has a rather narrow range of good growth—from 7.4-7.8, with the optimum rather definitely at 7.6. This is in agreement with Gates[11] who states that "a reaction favorable to the meningococcus cannot be determined from the total titrable acidity (phenolphthalein system) but depends solely upon the hydrogen-ion concentration of the medium." He states that in a serum dextrose broth, the range lies between 6.1 and 7.8, the optimum at 7.4.

*M. Melitensis.*—It does not produce luxuriant colonies except between $P_H$'s of 6.3 and 8.4, the optimum being between 6.6 and 8.0.

*Gonococci.*—Even those strains that have been a long time on artificial mediums, have rather well defined limits of growth, and rather sharply defined optimum. One of many batches, of Vedder's starch agar, that seemed to grow those gonococci exceptionally well, had a $P_H$ of 7.5. Subsequently, 4 batches were made, soluble starch, prepared by Small's method[12] being used, and set to $P_H$ 7.8, —8, —8.2, —8.4. These were autoclaved at 10 lbs. for 20 minutes, and yielded titrations of 7.6, —8, —8.2, —8.4. While the latter 3 batches produced growth, the 7.6 agar was far superior. Readings were made after 36 hours, the tubes melted, along with the growth, and $p_H$ estimations were made. The reaction had not changed appreciably.

*Indicators.*—The number of color indicators to denote acid production by bacteria is now becoming legion. Two well-known popular ones are rightly so, as far as true record of acid produced is concerned. Litmus and Andrade, both frequently used, are excellent indicators of the true neutral point, and slight acid production.

Litmus at a $P_H$ of 6.6-6.8 presents a reddish violet, at 7 it is a delicate lavender, and at 7.2-7.4 it is bluish violet.

Andrade at 7.4 is quite colorless, at 7.2 and 7 it is very faint pink, while at 6.8 and 6.6 the magenta is very definite.

[10] Hitchins, A. Parker: Personal communication.

[11] Jour. Exper. Med., 1919, 29. p. 321.

[12] Jour. Am. Chem. Soc., 1919, 41, p. 1.

*Peptones.*—There is a wide variation in reaction among the peptones in common use; there is even a variation between batches of the same brand. Of several samples titrated in a 1% solution, we find Witte 7, Armour's 6.6, Difco 7.3 and Fairchild's 4.9. These same samples, titrated cold, with phenolphthalein as indicator, yield the following: Witte 0.4% acid, Armour 0.7% acid, Difco 0.3% acid, and Fairchild 1.25% acid.

*Sugars.*—It might be of interest to know that 3 samples of lactose, in distilled water, sterilized by Arnold and autoclave, yield the following results: Sample 1 received 1 hour in Arnold on 3 consecutive days; sample 2 received in autoclave 15 lbs., 30 minutes; sample 3 was unheated. All three samples had a $P_H$ of 4.2. On boiling the tubes vigorously to remove carbon dioxid, the 3rd sample returned to neutral, or $P_H$ 7, the first 2 heated samples remained unchanged. Sterilization seems to produce some permanent change in reaction, though glucose was absent in these specimens.

TABLE 1
STANDARD SALT SOLUTIONS

| | | | | |
|---|---|---|---|---|
| pH 6.6 | 50 c c m/5 $KH_2PO_4$ | | 17.80 c c m/5 NaOH | Dilute to 200 c c |
| 6.8 | 50 c c m/5 $KH_2PO_4$ | | 23.65 c c m/5 NaOH | Dilute to 200 c c |
| 7.0 | 50 c c m/5 $KH_2PO_4$ | | 29.63 c c m/5 NaOH | Dilute to 200 c c |
| 7.2 | 50 c c m/5 $KH_2PO_4$ | | 35.00 c c m/5 NaOH | Dilute to 200 c c |
| 7.4 | 50 c c m/5 $KH_2PO_4$ | | 39.50 c c m/5 NaOH | Dilute to 200 c c |
| 7.6 | 50 c c m/5 $KH_2PO_4$ | | 42.80 c c m/5 NaOH | Dilute to 200 c c |
| 7.8 | 50 c c m/5 $KH_2PO_4$ | | 45.20 c c m/5 NaOH | Dilute to 200 c c |
| 8.0 | 50 c c m/5 $KH_2PO_4$ | | 46.80 c c m/5 NaOH | Dilute to 200 c c |
| 8.2 | 50 c c m/5 $H_3BO_3$ | m/5 KCl | 5.90 c c m/5 NaOH | Dilute to 200 c c |
| 8.4 | 50 c c m/5 $H_3BO_3$ | m/5 KCl | 8.50 c c m/5 NaOH | Dilute to 200 c c |

TABLE 2
REACTIONS OF CULTURE MEDIUMS FOR VARIOUS ORGANISMS

| Medium and Organism | Acid Limit | Alkaline Limit | Optimum |
|---|---|---|---|
| Russell double sugar for B. typhosus, B. paratyphosus A and B, dysenteriae and col | 7.0 | 7.8 | 7.4–7.6 |
| Endo's medium for intestinal flora | ... | ... | 7.8–8.0 |
| Brilliant green | 6.4 | 7.2 | 6.8–7.0 |
| Simple nutrient agar for typhoid | 4.0 | 9.6+ | 6.2–7.2 |
| B. paratyphosus A | 4.0 | 9.6+ | 6.2–7.2 |
| B. paratyphosus B | 4.0 | 9.6+ | 6.2–7.2 |
| Dysentery, Shiga | 4.8 | 9.6+ | 6.2–8.4 |
| Dysentery, Flexner | 4.8 | 9.6+ | 6.2–8.4 |
| Dysentery, "Y" | 4.8 | 9.6+ | 6.2–8.4 |
| Cholera vibrio | 5.6 | 9.6+ | 6.2–9.0 |
| M. melitensis | 6.3 | 9.6 | 6.6–8.2 |
| Infusion broth for pneumococcus | 5.0 | 8.0 | 7.8 |
| Streptococcus hemolyticus | 4.5 | 8.0 | 7.6–7.8 |
| S. viridans | 4.5 | 8.0 | 7.6–7.8 |
| Infusion glucose agar for meningococcus | 7.4 | 7.8 | 7.6 |
| Chocolate medium for B. influenzae | ? | ? | 7.8 |
| Vedder's starch medium for gonococcus | 7.0 | 8.0 | 7.4–7.6 |

## CONCLUSION

The adjustment of bacteriologic culture mediums according to hydrogen-ion concentration, because of its accuracy and simplicity, should wholly supplant the phenolphathalein (total acidity) method.